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Article *in* Natural Resources for Human Health · December 2022

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Received 09 August 2022
Revised 26 October 2022
Accepted 11 November 2022
Available online 11 December 2022

Edited by Si Mi

KEYWORDS:
Antidiabetic activity
Antioxidant inhibition
Chenopodium ambrosioides
GC-FID
GC-MS

Natr Resour Human Health 2023; 3 (2): 218-227
https://doi.org/10.53365/nrfhh/156457
eISSN: 2583-1194

GC-FID and GC-MS profiling and *in vitro* antidiabetic and antioxidant activities of *Chenopodium ambrosioides* L.

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ABSTRACT: *Chenopodium ambrosioides* is widely used in traditional medicines to manage several health conditions. This study aimed to investigate the chemical composition of the *n*-hexane fraction and the *in vitro* antidiabetic and antioxidant properties of *C. ambrosioides* L. The chemical composition was determined using Gas Chromatography-Flame Ionization Detection and Gas Chromatography-Mass Spectrometry. *In vitro* evaluations were assessed by evaluating the inhibitory potentials on the activities of α-glucosidase and antioxidant. A total of 58 phytochemicals were identified belonging to 11 classes of substances, of which aliphatic hydrocarbons (38.25%), diterpenes (20.54%), esters (16.33%), triterpenes (11.91%), diverse functional groups (3.74%), aromatic hydrocarbons (2.64%), sesquiterpenes (2.31%), alcohols (1.41%), ketones (0.29%), monoterpenes (0.16%), and fatty acids (0.14%). The major compounds were heptacosane (30.48%) (**46**), phytol (20.94) (**35**), and squalene (11.07%) (**56**). The methanol extract and its fractions showed moderate α-glucosidase activity, but their IC_{50} values were lower than the positive control 1-deoxynojirimycin. However, the methanol and methanol-water fractions exhibited more scavenging activity on 2,2-diphenyl-1-picrylhydrazyl with IC_{50} values similar to butylated hydroxyanisole (BHA). The plant is rich in various phytoconstituents, and its α-glucosidase and antioxidant status may justify its use in traditional medicine, especially for preventing complications of diabetes.

1. INTRODUCTION

Medicinal herbs are an essential source of natural compounds used as remedies for various diseases. The empirical knowledge of the beneficial potential of medicinal plants was transmitted over the centuries within each human community. They constitute the most ancient form of treatment for human and veterinary ailments used for thousands of years in traditional medicine in several countries worldwide (Marrelli, 2021). Medicinal plants contain various bioactive components, including alkaloids, carotenoids, glycosides, flavonoids, polysaccharides, saponins, terpenoids, etc., with antidiabetic potential (Przeor, 2022). The phytochemical composition and the health-beneficial effects of many medicinal plants have not yet been or still need to be more deeply studied (Kasali et al., 2022). Of 400,000 estimated plant species, only 6% have

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been investigated, and phytochemical studies of 15% have been carried out (Muhammad et al., 2021).

In several countries, including the Democratic Republic of Congo, the plant is locally used to treat Type-2 diabetes mellitus (Masunda et al., 2019), a severe health problem and global health pandemic. According to a current report, the worldwide incidence of diabetes mellitus increased by 102.9% in 2017 (Liu et al., 2020). Hyperglycemia is associated with excess free radical production resulting in oxidative stress. It is a critical parameter in diabetic complications by producing free radicals (Nguelefack et al., 2020). In this last decade, scientific efforts have been made to develop and design antidiabetic agents with hypoglycemic and antioxidant potentials with lower side effects. Over 1,000 plant species are being used to treat type-2 diabetes mellitus worldwide. More than 800 species of plants showing hypoglycemic activity can be essential sources for discovering and developing new types of antidiabetic molecules (Patel et al., 2012; Trojan-Rodrigues et al., 2012). Existing α-glucosidase drugs such as acarbose, miglitol, and voglibose have various digestive side effects and have no antioxidant properties.

Chenopodium ambrosioides L. [*Dysphania ambrosioides* (L.) Mosyakin & Clemants)] belongs to the family of the Amaranthaceae and is widely cultivated all over the world. The World Health Organization (WHO) pointed out that *C. ambrosioides* is among the most used plants in traditional medicines worldwide (Sá et al., 2016).

To our knowledge, no studies exist regarding the chemical profiling of the n-hexane fraction from leaf extract of *C. ambrosioides* and α-glucosidase evaluation *in vitro*. According to the literature, the antioxidant status has been evidenced only in crude extracts and essential oil (Kasali et al., 2021).

This study aims to identify different phytochemicals in the n-hexane fraction and investigate the *in vitro* antidiabetic and antioxidant properties of *C. ambrosioides*.

2. MATERIAL AND METHODS

2.1. Plant material collection and identification

Fresh leaves of *C. ambrosioides* were collected in Bukavu city, located in the eastern part of the Democratic Republic of Congo, between April and October 2019. Plant materials were identified and authenticated by the Department of Biology of "Centre de Recherche en Sciences Naturelles CRSN/Lwiro", and voucher specimens deposed under number LWI563359346.

2.2. Preparation of leaf the methanol extract

The leaves were air-dried at room temperature and then manually grounded to fine powders (Mowla et al., 2009; Tafesse et al., 2017). According to this protocol, the leaf powder (1.144 kg) was repeatedly extracted with the methanol in an Erlenmeyer flask by occasional shaking and stirring. The different obtained extracts were concentrated on a rotary evaporator (at 40-50°C) to obtain the crude quote (232.99 gr: yield 20.4%).

2.3. Fractionation of C ambrosioides methanol extract using Vacuum Liquid Chromatography

The methanol extract was subjected to vacuum liquid chromatography (VLC) on silica gel using the n-hexane, n-hexane-dichloromethane (1:1), dichloromethane, dichloromethane-methanol (1:1), methanol, and methanol-water (9:1) as the mobile phases, respectively. These sub-fractions were freed of solvents on rotavapor and further dried in the fuming hood for one week before submitting pharmacological studies.

2.4. Identification of phytochemicals by GC-FID and GC-MS

Gas Chromatography equipped with flame ionization detector (FID), capillary column SPB-5 was used. The experimental mass spectra of the volatile compounds were compared with the electronic mass spectral data reported in the literature (NIST database) for the identification of compounds (Khan et al., 2016; Wang et al., 2018). ChemDraw Ultra 8.0 software was used for drawing materials' structures.

2.5. Alpha-glucosidase Inhibition Assay

The enzyme inhibition assay is based on the breakdown of the substrate to produce a colored product, followed by measuring the absorbance (Kurihara et al., 1994).

2.6. Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method described by Gulcin et al. (Gülçin et al., 2005).

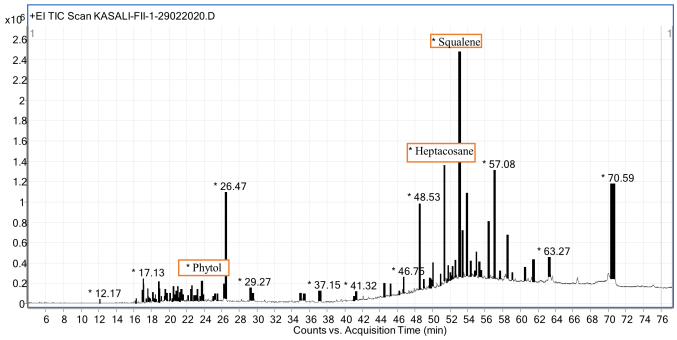

Figure 1. Typical chromatogram of chemical compounds present in the n-hexane fraction of *C.ambrosioides*

3. RESULTS AND DISCUSSION

3.1. Phytochemical identification

Figure 1 indicates a typical chromatogram of chemical compounds present in the n-hexane fraction of *C. ambrosioides*. However, Figure 2, 3 and 4 shows the structures of all compounds identified in the plant. A total of 58 phytoconstituents were identified by GC and GC–MS analysis (Table 1).

Identified compounds were grouped in 11 classes of substances, including aliphatic hydrocarbons (35.54%), diterpenes

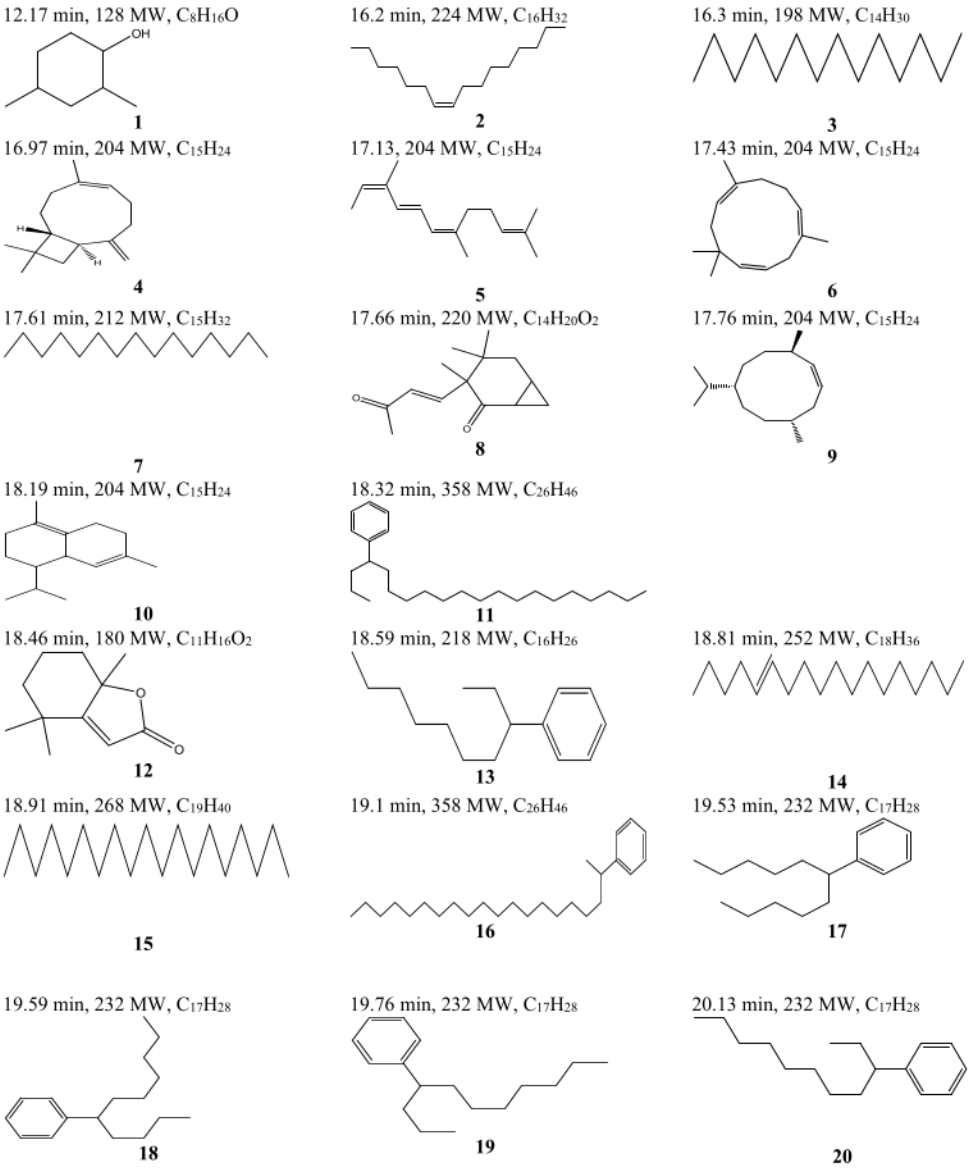

Figure 2. Phytoconstituents (**1**-**20**) identified in the n-hexane fraction from *C. ambrosioides* methanol extract

(20.94%), esters (15.17%), triterpenes (11.07%), bromine-containing (7.05%), diverse functional groups (3.76%), aromatic hydrocarbons (2.45%), sesquiterpenes (2.15%), alcohols (1.31%), ketones (0.27%), monoterpenes (0.15%), and fatty acids (0.13%). The main compounds were heptacosane (30.48%), phytol (20.94), and squalene (11.07%).

Cyclohexanol, 2,4-dimethyl- (**1**), (Z)-7-hexadecene (**2**), tetradecane (**3**), caryophyllene (**4**), (Z)-β-farnesene (**5**), α-caryophyllene (**6**), pentadecane (**7**); 3,4,4-trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one (**8**), germacrene D (**9**), cadina-1(10),4-diene (**10**), benzene, (1-propylheptadecyl)- (**11**), dihydroactinidiolide (**12**), benzene, (1-ethyloctyl)- (**13**), (E)-5-octadecene (**14**), nonadecane (**15**), benzene, (1-methylnonadecyl)- (**16**), benzene, (1-pentylhexyl)- (**17**), benzene, (1-butylheptyl)- (**18**), benzene, (1-propyloctyl)- (**19**), benzene, (1-ethylnonyl)- (**20**), 1-decanol, 2-hexyl- (**21**), heptadecane (**22**), tetradecane, 2,6,10-trimethyl- (**23**), benzene, (1-methyldecyl)- (**24**), methyl tetradecanoate (**25**), cyclohexane, 1,1,3-trimethyl-2-(3-methylpentyl)- (**26**), benzene, (1-pentylheptyl)- (**27**), β-Guaiene (**26**), benzene, (1-propylnonyl)- (**29**), benzene, (1-ethyldecyl)- (**30**), 1-nonadecene (**31**), dodecane, 2-phenyl- (**32**), isopropyl myristate (**33**), benzene, (1-pentyloctyl)- (**34**), phytol (**35**), 2-pentadecanone, 6,10,14-trimethyl- (**36**), (7a-isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol (**37**), (Z)-7-hexadecenoic acid, methyl ester (**38**), hexadecanoic acid, methyl ester (**39**), hexadecanoic acid, ethyl ester (**40**), heneicosane (**41**), 9,12-octadecadienoic acid, methyl ester (**42**), (Z)-9-octadecenoic acid, methyl ester (**43**), octadecanoic acid, methyl ester (**44**), octadecanoic acid, ethyl ester (**45**), heptacosane (**46**)

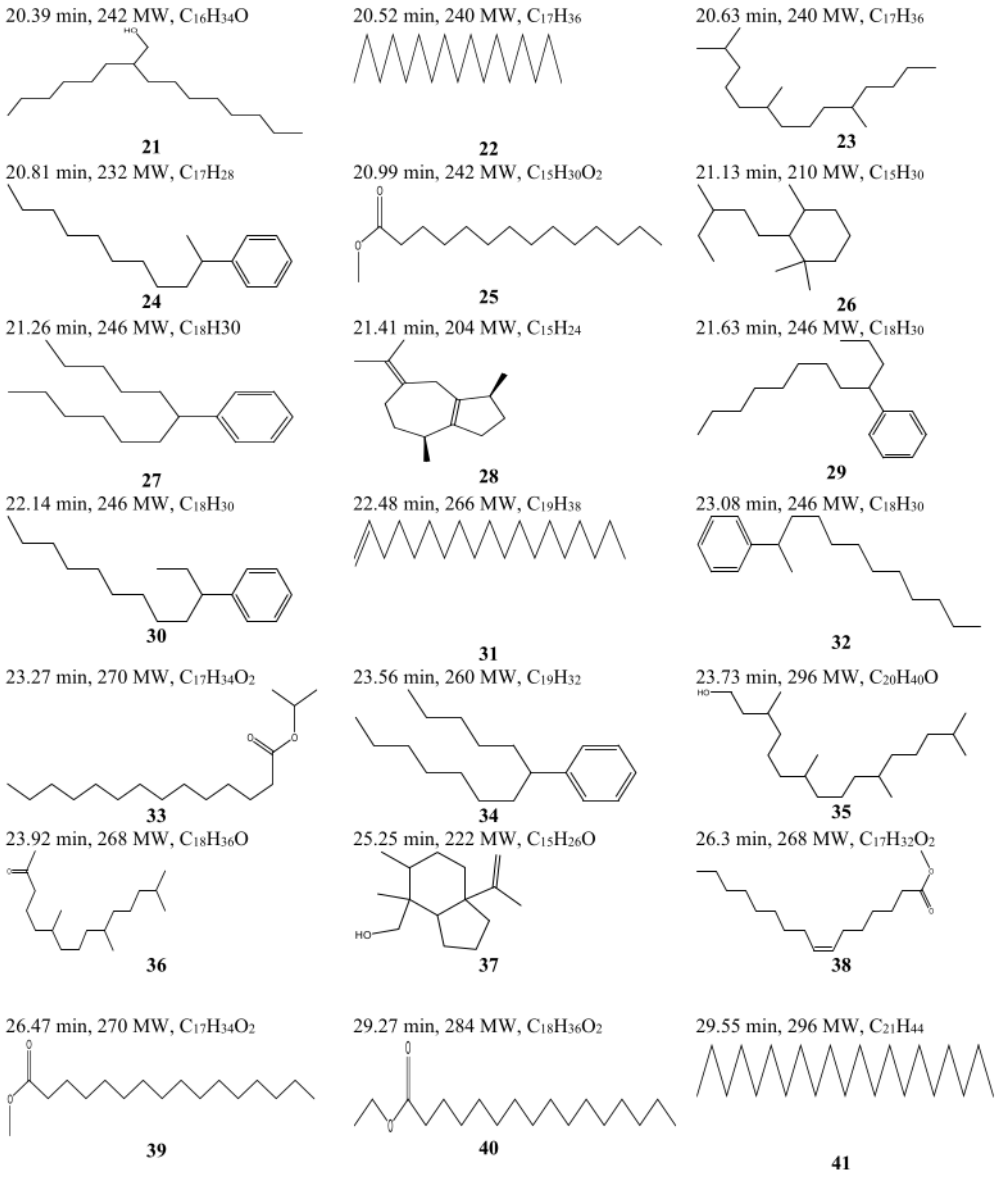

Figure 3. Phytoconstituents (**21**-**41**) identified in the n-hexane fraction from *C. ambrosioides* methanol extract

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eicosanoic acid, methyl ester (**47**), 7-methyl-Z-tetradecen-1-ol acetate (**48**), trans-13-Octadecenoic acid (**49**), 17-octadecynoic acid, methyl ester (**50**), (12-Methyl-E,E-2,13-octadecadien-1-ol (**51**), oleic acid (**52**), ethyl iso-allocholate (**53**), oleic acid, 3-(octadecyloxy)propyl ester (**54**), squalene (**55**), ethanol, 2-(octadecyloxy)- (**56**), Z-(13,14-epoxy)tetradec-11-en-1-ol acetate (**57**), and ethanol, 2-(9-octadecenyloxy)-, (Z)- (**58**).

Previous studies have reported some compounds extracted from the leaves, mainly in pentane and essential oil. In this present study, we report the chemical composition of the n-hexane fraction of methanolic extract of leaves, showing 58 phytochemicals belonging to 11 classes of substances. Those compounds include α-guaiene (Sagrero-Nieves & Bartley, 1995), α-caryophyllene and caryophyllene (Gbolade et al., 2010; Gillij et al., 2008; Jaramillo et al., 2012), squalene (Reyes-Becerril et al., 2019), phytol (Jaramillo et al., 2012), dihydroactinidiolide (Reyes-Becerril et al., 2019); 3,7,11,15-tetramethyl-2-hexadecen-1-ol and 1-nonadecene (Mostafa et al., 2016); and 9,12-octadecadienoic acid, methyl ester (Reyes-Becerril et al., 2019). Essential oils are a complex mixture of volatile plant compounds composed of terpenoids (mainly monoterpenes and sesquiterpenes) and phenolic compounds. The essential oil's chemical composition is highly variable from plant to plant, even in the same species, related to different factors (abiotic, biotic, methods of extraction, conservation, and postharvest conditions) (Mkaddem et al., 2022). Although the n-hexane fraction of the methanolic extract is far from essential oil, these results show the presence of a good number of terpene

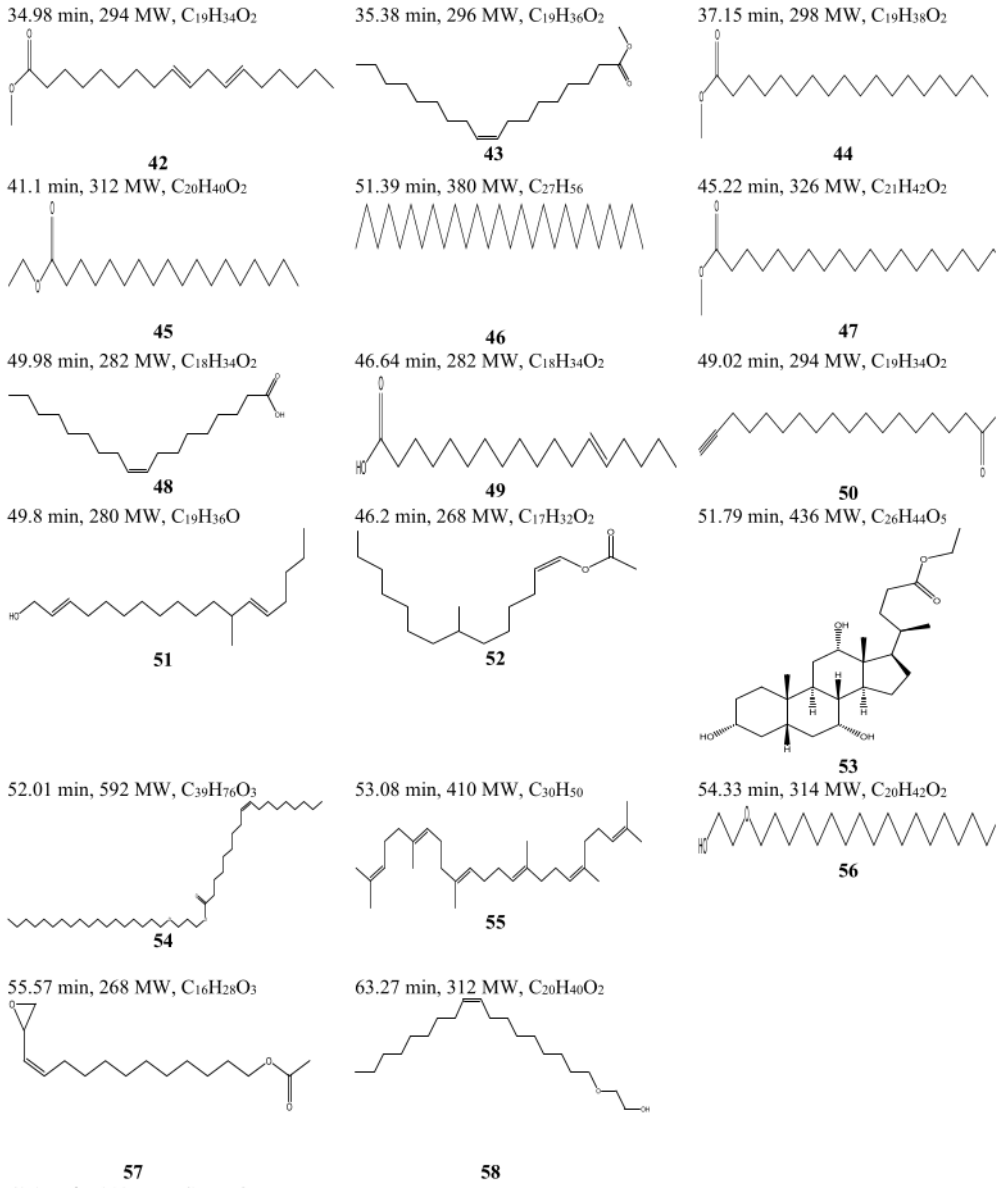

Figure 4. Phytoconstituents (**42**-**58**) identified in the n-hexane fraction from *C. ambrosioides* methanol extract

compounds.

On the other hand, our study showed a few phytoconstituents close to those identified by other authors. For example, germacrene D, hexadecanoic, and octadecanoic acids were identified in our sample with their methyl and ethyl esters. Germacrene D-4-ol (Gillij et al., 2008), hexadecanoic acid (Pino et al., 2003), and octadecanoic acid (Shah & Khan, 2017) without their esters were identified in essential and the methanol (ethyl acetate) extract. In the line of our results, tetradecane, caryophyllene oxide, hexadecanoic acid, caryophyllene, germacrene D, 9, 12-octadecadienoic acid, methyl ester, oleic acid, phytol, tetradecane, squalene, heneicosane, and methyl derivatives have been identified by GC-MS analysis in the n-hexane fraction/extract of different plant species (Godwin et al., 2015; Govindarajan et al., 2016; Ivanov et al., 2018; Nadaf et al., 2012). It has been observed that in the *n*-hexane fraction or extract of different plants, there is a remarkable variability of compounds, particularly the methyl esters. According to the literature, the methyl esters are possible artifacts due to the extraction with methanol (Venditti, 2018).

Forty-six out of sixty-one phytoconstituents are reported for the first time by the plant. Based on literature data, approximately 330 compounds (including their isomers) have been identified in different extracts, fractions of *C. ambrosioides*, and the majority (59.54%) mainly in essential oil (Kasali et al., 2021). However, contrary to our results, a chemical investigation of the n-hexane extract from Brazilian *C. ambrosioides* showed the presence of seven monoterpenes, include α-terpinene, p-cymene, benzyl alcohol (Z)-ascaridole, carvacrol, and (E)-ascaridole (Jardim et al., 2010).

Table 1
Phytoconstituents identified in the *n*-hexane fraction from *C. ambrosioides* methanol extract

Name of the compound	Class	Molecular formula	Molecular weight
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	Alcohols	$C_{15}H_{26}O$	222
(E)-5-Octadecene	Aliphatic hydrocarbons	$C_{18}H_{36}$	252
(Z)-7-Hexadecene	Aliphatic hydrocarbons	$C_{16}H_{32}$	224
(Z)-7-Hexadecenoic acid, methyl ester	Esters	$C_{17}H_{32}O_2$	268
(Z)-9-Octadecenoic acid, methyl ester	Esters	$C_{19}H_{36}O_2$	296
(Z)-β-Farnesene	Sesquiterpenes	$C_{15}H_{24}$	204
12-Methyl-E,E-2,13-octadecadien-1-ol	Alcohols	$C_{19}H_{36}O$	280
17-Octadecynoic acid, methyl ester	Esters	$C_{19}H_{34}O_2$	294
1-Decanol, 2-hexyl-	Alcohols	$C_{16}H_{34}O$	242
1-Nonadecene	Aliphatic hydrocarbons	$C_{19}H_{38}$	266
2-Pentadecanone, 6,10,14-trimethyl-	Ketones	$C_{18}H_{36}O$	268
3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one	Ketones	$C_{14}H_{20}O_2$	220
7-Methyl-Z-tetradecen-1-ol acetate	Esters	$C_{17}H_{32}O_2$	268
9,12-Octadecadienoic acid, methyl ester	Esters	$C_{19}H_{34}O_2$	294
Benzene, (1-butylheptyl)-	Aromatic hydrocarbons	$C_{17}H_{28}$	232
Benzene, (1-ethyldecyl)-	Aromatic hydrocarbons	$C_{18}H_{30}$	246
Benzene, (1-ethylnonyl)-	Aromatic hydrocarbons	$C_{17}H_{28}$	232
Benzene, (1-ethyloctyl)-	Aromatic hydrocarbons	$C_{16}H_{26}$	218
Benzene, (1-methyldecyl)-	Aromatic hydrocarbons	$C_{17}H_{28}$	232
Benzene, (1-methylnonadecyl)-	Aromatic hydrocarbons	$C_{26}H_{46}$	358
Benzene, (1-pentylheptyl)-	Aromatic hydrocarbons	$C_{18}H30$	246
Benzene, (1-pentylhexyl)-	Aromatic hydrocarbons	$C_{17}H_{28}$	232
Benzene, (1-pentyloctyl)-	Aromatic hydrocarbons	$C_{19}H_{32}$	260
Benzene, (1-propylheptadecyl)-	Aromatic hydrocarbons	$C_{26}H_{46}$	358
Benzene, (1-propylnonyl)-	Aromatic hydrocarbons	$C_{18}H_{30}$	246
Benzene, (1-propyloctyl)-	Aromatic hydrocarbons	$C_{17}H_{28}$	232
Cadina-1(10),4-diene	Sesquiterpenes	$C_{15}H_{24}$	204
Caryophyllene	Sesquiterpenes	$C_{15}H_{24}$	204
Cyclohexane, 1,1,3-trimethyl-2-(3-methylpentyl)-	Aliphatic hydrocarbons	$C_{15}H_{30}$	210
Cyclohexanol, 2,4-dimethyl-	Alcohols	$C_8H_{16}O$	128
Dihydroactinidiolide	Monoterpenes	$C_{11}H_{16}O_2$	180
Dodecane, 2-phenyl-	Aromatic hydrocarbons	$C_{18}H_{30}$	246
Eicosanoic acid, methyl ester	Esters	$C_{21}H_{42}O_2$	326
Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Diverse functional groups	$C_{20}H_{40}O_2$	312
Ethanol, 2-(octadecyloxy)-	Diverse functional groups	$C_{20}H_{42}O_2$	314
Ethyl iso-allocholate	Esters	$C_{26}H_{44}O_5$	436
Germacrene D	Sesquiterpenes	$C_{15}H_{24}$	204
Heneicosane	Aliphatic hydrocarbons	$C_{21}H_{44}$	296
Heptacosane	Aliphatic hydrocarbons	$C_{27}H_{56}$	380
Heptadecane	Aliphatic hydrocarbons	$C_{17}H_{36}$	240
Hexadecanoic acid, ethyl ester	Esters	$C_{18}H_{36}O_2$	284
Hexadecanoic acid, methyl ester	Esters	$C_{17}H_{34}O_2$	270

Continued on next page

Table 1 continued

Isopropyl myristate	Esters	$C_{17}H_{34}O_2$	270
Methyl tetradecanoate	Esters	$C_{15}H_{30}O_2$	242
Nonadecane	Aliphatic hydrocarbons	$C_{19}H_{40}$	268
Octadecanoic acid, ethyl ester	Esters	$C_{20}H_{40}O2$	312
Octadecanoic acid, methyl ester	Esters	$C_{19}H_{38}O_2$	298
Oleic acid	Fatty acids	$C_{18}H_{34}O_2$	282
Oleic acid, 3-(octadecyloxy)propyl ester	Diverse functional groups	$C_{39}H_{76}O_3$	592
Pentadecane	Aliphatic hydrocarbons	$C_{15}H_{32}$	212
Phytol	Diterpenes	$C_{20}H_{40}O$	296
Squalene	Triterpenes	$C_{30}H_{50}$	410
Tetradecane	Aliphatic hydrocarbons	$C_{14}H_{30}$	198
Tetradecane, 2,6,10-trimethyl-	Aliphatic hydrocarbons	$C_{17}H_{36}$	240
Trans-13-Octadecenoic acid	Fatty acids	$C_{18}H_{34}O_2$	282
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	Diverse functional groups	$C_{16}H_{28}O_3$	268
α-Caryophyllene	Sesquiterpenes	$C_{15}H_{24}$	204
β-Guaiene	Sesquiterpenes	$C_{15}H_{24}$	204

3.2. *In vitro* pharmacological evaluations

Table 2 reports the *in vitro* antidiabetic (α-glucosidase) and antioxidant investigations of the leaf methanol extract and its fractions.

Table 2
In vitro α-glucosidase and DPPH inhibitions of the methanol extract and their fractions

No.	Extract/Fractions	α-glucosidase inhibition	Antioxidant activity
1	MECa	36.7± 0.83	61.4± 0.22
2	F1	29.8± 0.83	72.1±0.44
3	F2	25.4± 0.82	79.3± 0.39
4	F3	23.7± 0.84	85.2± 0.18
5	F4	20.4± 0.72	67.7± 0.32
6	F5	22.2± 0.93	44.9 ± 0.07
7	F6	30.4± 0.33	48.8± 0.04
8	DNJ	3.9 ± 0.71	-
9	BHA	-	44.2 ± 0.77

All the values are represented as IC_{50} (μM). Data are expressed as the mean ± standard deviation (n=3). MECa (the methanol extract of *C. ambrosioides*); F1 (n-hexane); F2 (the *n*-hexane-dichloromethane); F3 (dichloromethane); F4 (dichloromethane-methanol); F5 (methanol); F6 (methanol-water); DNJ (1-deoxynojirimycin), IC_{50} values [the means (95% confidence interval) of three measurements]; BHA (Butylated hydroxyanisole).

The methanol extract and fractions demonstrated *in vitro* antidiabetic property by inhibiting α-glucosidase activity. According to their IC_{50} values, dichloromethane-methanol bit was the most effective (20.4± 0.72 μM), followed by the methanol fraction (22.2± 0.93 μM), the dichloromethane fraction (23.7± 0.84 μM), and the n-hexane-dichloromethane fraction (25.4± 0.82 μM).

On the other vein, fractions F5 (methanol) and F6 (methanol-water) showed the best antioxidant potential than crude extract and different fractions. Their IC_{50} values of 44.9 ± 0.07 and 48.8 ± 0.04 (μM), respectively, were close to the IC_{50} value of the standard drug (BHA).

According to our results (Table 2), all compounds showed antidiabetic potential, and according to the classification of the sample based on IC_{50} or CC_{50} (Indrayanto et al., 2021), they possess moderate activity. Nevertheless, the most potent fractions are located in the polarity range between the methanol and dichloromethane fractions. Several phytoconstituents can exist in that range of polarity, including steroids, glycosides, alkaloids, anthraquinones, tannins, flavonoids, phenolic acids, peptides, polysaccharides, etc. However, as natural α-glucosidase inhibitors, flavonoids, alkaloids, terpenoids, steroids, quinines, phenylpropanoids, anthocyanins, tannins, phenolics, curcuminoids, miscellaneous, are the most found (Kumar et al., 2011; Yin et al., 2014). Moreover, previous studies reported the inhibition effect of either the dichloromethane extract or fraction on α-glucosidase (Ferulago bracteata, Croton bonplandianum, Rhizophora apiculata, etc.). With IC_{50} of 3.9 ± 0.71 (μM), 1-deoxynojirimycin presented enzyme inhibition 9.2 times greater than the methanol extract and 5.2 times methanol-dichloromethane fraction. For example, similar to our results, the methanol extract of Ceiba pentandra inhibited 87.79% of α-glucosidase. However, acarbose (Drug standard) inhibited 10 times potent than that of the methanol extract (Nguelefack et al., 2020).

lso reported the intense antioxidant activity of the methanol and the methanol-water fractions of *C. ambrosioides* close to the standard drug (BHA). There is a high probability of finding flavonoids and their glucosides in these fractions. It is known that the polyphenolic compounds include flavonoids, are suitably extracted in hydroalcoholic solutions (De Luna et al., 2020). The best-described pharmacological potential of flavonoids is their antioxidant capacity, depending on functional groups' arrangement about the nuclear structure. Scavenging reactive oxygen species, upregulation or protection of antioxidant defenses, and suppressing their formation through enzyme inhibition and chelation of trace elements involved in a free radical generation are the primary antioxidant mechanisms of natural flavonoids (Kumar & Pandey, 2013).

4. CONCLUSION

The phytochemical composition of the n-hexane fraction of *C. ambrosioides* demonstrated that the plant possesses phytoconstituents from various groups, including fatty acids and esters, alcohols, and hydrocarbons aldehydes, ketones, diverse functional groups, and terpenes. All fractions produced moderate α-glucosidase inhibition, and the methanol and methanol-water fractions strongly inhibited the DPPH radical. In addition, this first *in vitro* investigation of the effect of the methanol extract and its fractions on α-glucosidase and scavenging activities exhibited the plant's potential, which justifies its traditional use as an antidiabetic drug. Pharmacological studies on diverse extracts and isolated compounds from the plant are necessary to exploit this plant properly.

ACKNOWLEDGMENTS

The authors are grateful to the Pharm-Bio Technology and Traditional Medicine Center (PHARMBIOTRAC) and Mbarara University of Science and Technology for support during the work.

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FUNDING

The World Academy of Science (TWAS) and the International Center for Chemical and Biological, the University

of Karachi (ICCBS) supported this work [grant number FR 3240305612]. The funding agent had no role in the writing of the present manuscript.

AUTHOR CONTRIBUTIONS

FMK - Research concept and design; FMK, JT - Collection and/or assembly of data; FMK, MSA, ML, RAO, GTT - Data analysis and interpretation; FMK - Writing the article; MSA, JNK, JT, ML, AGA - Critical revision of the article; FMK, MSA, JNK, JT, ML, RAO, AGA - Final approval of the article.

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